with reports of tuberculosis due to *M. bovis* in AIDS patients (Bouvet et al., 1993; O'Reilly et al., 1995) and with increasing incidence of tuberculosis globally, rapid and reliable diagnostic assays are required not only for detection but also identification of the pathogenic mycobacteria in clinical samples. This is essential for prompt diagnosis, treatment and control of tuberculosis. Identification of human pathogenic mycobacteria becomes all the more relevant with the need to develop alternate new generation vaccines for human use.

Immunogenecity of HupB protein: Two methods were used to identify mycobacterial constituents associated with human response namely the T cell blot and immuno – subtraction assays (Prabhakar et al., 1998). The 30kDa fraction of the mycobacterial lysate was found to induce the highest lymphoproliferative index among the tuberculin reactors. In immuno-subtractive assays a prominent reactive band was similarly seen at approximately 30kDa. The 30kDa protein was electro-eluted from the SDS-PAGE gel and purified to homogeneity.

Using the internal peptide sequence, Seq ID No. 6 (VKPTSVPAFRPGAQFK) a 100% identity was obtained with 16 amino acids of the cosmid CY349. The corresponding gene was later annotated and designated as the *hupB* gene (Rv 2986c, Cole et al., 1998). The protein was found to be localized in the cytoplasm and on the cytoplasmic surface of the mycobacterial membrane, by immuno-gold electron microscopy. The *hupB* gene has been classified among the DNA binding (histone like) proteins of *M. tuberculosis* (Cole et al., 1998). Primers were designed to amplify the *hupB* gene. A 645 bp amplicon was obtained in case of *M. tuberculosis*. The α^{32}P labeled PCR amplicon was used in Southern hybridization to establish the size, prevalence and organization of the *hupB* gene in members of the MTB complex (*M. tuberculosis* and *M. bovis*) and other mycobacterial species.

Analyzing and validating the size of the amplified fragments. Determining the complete sequence of the said amplified fragments. Inferring from the sequence whether it is *M. tuberculosis* or *M. bovis*.

Another embodiment is a method wherein the DNA probe consists of sequence ID No. 7 or sequence ID No. 8 or a complement thereof tagged with a detectable label.

Another embodiment is a method wherein the step of differentiation consists in determining the smaller size of the amplified fragment obtained from *Mycobacterium bovis*.

Another embodiment is a method wherein the PCR amplified fragment in *Mycobacterium bovis* was 618 bp.

Another embodiment is a method wherein the PCR amplified fragment in *Mycobacterium tuberculosis* was 645 bp.

Another embodiment is a method wherein the PCR amplified fragment in *Mycobacterium bovis* was 291 bp.

Another embodiment is a method wherein the PCR amplified fragment in *Mycobacterium tuberculosis* was 318 bp.

Another embodiment is a method wherein the PCR amplified fragment in *Mycobacterium bovis* was 89 bp.

Processing of bacilli for specificity analysis

All the mycobacterial and non-mycobacterial strains grown on solid media (LJ slants all mycobacterial species), LB agar (*E. coli*) nutrient agar (*Aspergillus niger*, *Nocardia asteriodes*, *Pseudomonas aeruoginosa*, *Klebsiella pneumoniae*) or blood agar (*Corynebacterium diphtheriae*, *Streptococcus pneumoniae*) were scraped with the help of sterile toothpicks and re-suspended in sterile distilled water containing 0.1% Triton X-100. Re-suspended bacilli were boiled at 100°C for 20 minutes and an aliquot (2µl) was used for PCR.

PCR Analysis:

1) 23S rDNA target: Primers: C, **Seq ID No.9**(5' gtgagcgacgggatttgcctat 3') and L, **Seq ID No. 10**(5' accacccaaaaccggatcgat 3') were used to detect the presence of DNA from organisms belonging to genus Mycobacterium. The expected size of the amplicon was 174bp (Verma et al., 1994; Dasgupta et al., 1998).

2) *hupB* DNA target : Primers **N, Seq ID No. 1** (5' ggagggttgggatgaacaaagcag 3') and **S, Seq ID No. 2** (5' gtatccgtgtgtcttgacctatttg 3') were used to amplify *hupB* gene sequences. The expected size of the amplicon was ~645 bp (Table II, Fig:1) in *M. tuberculosis* and 618 bp in *M. bovis*.

Each reaction (20µl) contained 1.5 mM $MgCl_2$, 0.5 µM of primers, 200 µM dNTPs, 10 mM Tris-HCl (pH 8.8 at 25°C), 50 mM KCl, 0.08% Nonidet P40, and 0.5 Units of Taq DNA Polymerase. The PCR reaction was subjected to initial denaturation at 94°C for 10 min., and 35 cycles of each of 1 min at 94°C, 1 min., at 63°C and 1 min at 72°C followed by final extension at 72°C for 30 mins. The fragments were analyzed on a 1.2 % agarose gel and stained with ethidium bromide.

The C-terminal portion of the gene was amplified by using **M, Seq ID No. 3** (5' gcagccaagaaggtagcgaa 3') with **S, Seq ID No. 2** (5' gtatccgtgtgtcttgacctatttg 3'), the expected amplicon was ~ 318 bp.

Nested PCR: DNA extracted from clinical samples / cultivated mycobacteria were processed for PCR with primers Seq.ID. No.1-N and Seq.ID. No.2-S . The PCR product obtained using the primers Seq.ID. No.1-N and Seq.ID. No.2-S was used as target DNA in nested PCR.

Each reaction (40µl) contained 2.5 mM $MgCl_2$, 0.5 µM of primers, 200 µM dNTPs, 10 mM Tris-HCl (pH 8.8 at 25°C), 50 mM KCl, 0.08% Nonidet P40, and 0.5 Units of Taq DNA Polymerase. The PCR reaction was subjected to initial denaturation at 95°C for 10 min., and 35 cycles of 1 min at 94°C, 1 min., and 30 seconds at 59°C and final extension at 72°C for 7 mins. The fragments were analyzed on a 3.5 % agarose gel / 8 % non-reducing polyacrylamide gel and stained with ethidium bromide. The C- terminal portion of the gene was also amplified by using Seq.ID. No.4-F (5' ccaagaaggcgacaaagg3') with Seq.ID. No.5-R (5' gacagctttcttggcggg3'), the expected amplicon was ~ 116 bp in case of *M.tuberculosis* and 89 bp in case of *M.bovis*, (Table II, Fig:1).

Southern Hybridization: The PCR amplicons resolved on the agarose gel were transferred on to nitro-cellulose membrane (Southern, 1975). The blots were then hybridized with α-^{32}P labeled 645 bp *hupB* (Seq ID No.6) gene probe from *M. tuberculosis*, (*Pst*I & *Nco*I digest from the plasmid pHLPMT / probe generated by PCR using **N** (Seq ID No.1-N) and **S** (Seq ID No.2-S) primers and *M. tuberculosis*, DNA.).

Restriction Fragment Length Polymorphism:

SequenceName : seq id no. 6

OrganismName : hup B - *M. tuberculosis*, Rv2986c, Accession No. P95109

atgaacaaag cagagctcat tgacgtgctc acacagaaat tgggctcgga ccgtcggcag 60
gcgaccgccg ccgtcgagaa tgtcgttgac acgattgtgc gtgcggtaca caaaggcgac 120
agcgtcacca ttaccgggtt cggtgtgttc gaacagcgtc gccgcgcggc tcgagtggcc 180
cgcaatccgc gtaccggcga gacagtaaag gtgaagccga cgtcggtgcc ggcgttccgc 240
ccgggcgcgc aattcaaagc ggttgtgtct ggcgcgcagc gtctcccggc agaaggaccc 300
gctgttaagc gtggtgtggg ggccagtgca gccaagaagg tagcgaagaa ggcacctgcc 360
aagaaggcga caaaggccgc caagaaggcg gcgaccaagg cgcccgccag gaaggcggcg 420
accaaggcgc ccgccaagaa agcggcgacc aaggcgcccg ccaagaaagc tgtcaaggcc 480
acgaagtcac ccgccaagaa ggtgaccaag gcggtgaaga agaccgcggt caaggcatcg 540
gtgcgtaagg cggcgaccaa ggcgccggca aagaaggcag cggccaagcg gccggctacc 600
aaggctcccg ccaagaaggc aaccgctcgg cggggtcgca aatag 645

SequenceName : Seq Id no.7

OrganismName : Hlp of *Mycobacterium bovis*, Accession No. Y18421

atgaacaaag cagagctcat gacgtgctc acacagaaat tgggctcgga ccgtcggcag 60
gcgaccgccg ccgtcgagaa tgtcgttgac acgattgtgc gtgcggtaca caaaggcga 120
agcgtcacca ttaccgggtt cggtgtgttc gaacagcgtc gccgcgcggc tcgagtggcc 180
cgcaatccgc gtaccggcga gacagtaaag gtgaagccga cgtcggtgcc ggcgttccgc 240
ccgggcgcgc aattcaaagc ggttgtgtct ggcgcgcagc gtctcccggc agaaggaccc 300
gctgttaagc gtggtgtggg ggccagtgca gccaagaagg tagcgaagaa ggcacctgcc 360
aagaaggcga caaaggccgc caagaaggcg gcgaccaagg cgcccgccaa gaaagcggcg 420
accaaggcgc ccgccaagaa agctgtcaag gccacgaagt cacccgccaa gaaggtgacc 480
aaggcggtga agaagaccgc ggtcaaggca tcggtgcgta aggcggcgac caaggcgccg 540
gcaaagaagg cagcggccaa gcggccggct accaaggctc ccgccaagaa ggcaaccgct 600
cggcggggtc gcaaatag 618

We Claim:

1. Oligonucleotide primers for specific amplification of the *hupB* gene of *Mycobacterium* species selected from the group consisting of Seq ID No. 1, Seq ID No. 2, Seq ID No. 3, Seq ID No. 4, Seq ID No. 5.
2. A method for differentiating *Mycobacterium* species based on target gene encoding for histone like proteins such as *hup B* comprising of :
    a) Obtaining DNA from culture or from clinical samples.
    b) Amplifying a part of the target gene encoding for histone like proteins such as *hup B* of *Mycobacterium* species using said DNA as a template in a polymerase chain reaction with a pair of oligonucleotide primers according to claim 1.
    c) Detecting said amplified fragment of the *hup B* gene to detect the presence of *Mycobacterial* species or not and differentiating *Mycobacterium tuberculosis* from *Mycobacterium bovis* based on the size of the amplified fragment.
3. A method according to claim 2a, wherein said *Mycobacterium tuberculosis*, and or *Mycobacterium bovis* species is selected from a group of genetically related *Mycobacteria* and from unrelated microorganisms.
4. A method according to claim 2, wherein the pair of oligonucleotide primers comprises of Seq ID No. 1 and Seq ID No. 2.
5. A method according to claim 2, wherein the pair of oligonucleotide primers comprises of Seq ID No. 3 and Seq ID No. 2.
6. A method according to claim 2, wherein the pair of oligonucleotide primers comprises of Seq ID No. 4 and Seq ID No. 5.

7. A method of claim 2c, wherein the amplified fragments are detected by ethidium bromide staining or DNA probe hybridization.

8. A method as claimed in claim 2, wherein the step of differentiating comprising the steps of :

a) Designing a set of primers according to claim 1, Seq ID No. 1, Seq ID No. 2, Seq ID No. 3, Seq ID No. 4, Seq ID No. 5, to amplify a part of the said *hup B* gene from *Mycobacterium tuberculosis* and *Mycobacterium bovis*.

b) Obtaining DNA from culture or from clinical samples.

c) Amplifying a part of the target gene encoding for histone like proteins such as *hup B* of *Mycobacterium* species using said DNA as a template in a polymerase chain reaction with a pair of oligonucleotide primers according to claim 1.

d) Analyzing and validating the size of the amplified fragments.

e) Determining the complete Sequence of the said amplified fragments.

f) Inferring from the sequence whether it is *M. tuberculosis* or *M. bovis*.

9. A method according to claim 7 wherein the DNA probe consists of sequence ID No. 7 or sequence ID No. 8 or a complement thereof tagged with a detectable label.

10. A method as claimed in claim 2 wherein the step of differentiation consists in determining the smaller size of the amplified fragment obtained from *Mycobacterium bovis*.

11. A method according to claim 4 wherein the PCR amplified fragment in *Mycobacterium bovis* was 618 bp.

12. A method according to claim 4 wherein the PCR amplified fragment in *Mycobacterium tuberculosis* was 645 bp.

13. A method according to claim 5 wherein the PCR amplified fragment in *Mycobacterium bovis* was 291 bp.

14. A method according to claim 6 wherein the PCR amplified fragment in *Mycobacterium tuberculosis* was 318 bp.

15. A method according to claim 5 wherein the PCR amplified fragment in *Mycobacterium bovis* was 89 bp.

16. A method according to claim 5 wherein the PCR amplified fragment in *Mycobacterium tuberculosis* was 116bp.

17. A method according to claim 2 wherein the PCR amplified fragment in *Mycobacterium bovis* was 27 bp smaller than that of *Mycobacterium tuberculosis* .

18. A method as claimed in 2 wherein differentiating *M. tuberculosis* and *M. bovis* comprising the steps of :

    a) Amplifying a part of the target *hup B* gene from *M. tuberculosis* and *M. bovis* in a polymerase chain reaction with primers Seq. ID No.1 and Seq. ID No.2

    b) Restricting the amplified fragment with *Hpa II* restriction enzyme to produce restricted fragments.

    c) Separating the restricted fragments by electrophoresis on 12% polyacrylamide gel

    d) Detecting the restricted fragments by staining with ethidium bromide.

19. A method according to claim 18 wherein the restricted fragment in *M. tuberculosis* was 280 bp and 150 bp.

20 A method according to claim 18 wherein the restricted fragment in M. bovis was 253 bp and 150 bp.

21 *Hup B* gene (Seq ID No. 8) as claimed in 1 substantially as herein described .

22 A process as in preceding claims substantially as herein described

23 *Hup B* gene (Seq ID No. 7) as claimed in 1 substantially as herein described

24 A process as in preceding claims substantially as herein described.